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Scientific Article

Exogenous application of melatonin mitigates salt stress in soybean

Aplicação exógena de melatonina mitiga o estresse salino em soja

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ABSTRACT - Salinity is an abiotic factor that impairs the growth and physiological, biochemical, and molecular mechanisms of plants. Among plants, soybeans are an important crop worldwide, so managing abiotic factors is essential to mitigate plant damage. However, biostimulants, such as melatonin, are being employed to alleviate the stress caused by these factors. Therefore, this study aimed to evaluate the growth, photosynthetic pigments, and water relations of soybean plants subjected to salinity levels and exogenous melatonin application. The research was conducted in experimental area belonging to the Federal Rural University of the Semi-Arid Region, Mossoró, RN, Brazil. The experimental design was randomized blocks, arranged in a 3 x 3 factorial scheme (three salinity levels in the irrigation water – 0.50, 3.00, and 5.00 dS m^{-1} and three melatonin concentrations – 0, 0.5, and 1 mM) with three replications. At 47 days after planting, plant height, stem diameter, number of leaves, root length, chlorophyll content (a, b, and total), relative water content, leaf moisture, and electrolyte leakage were evaluated. Soybean plants tolerated the effects of salinity on growth aspects, photosynthetic pigments, and water relations up to 3.00 dS m^{-1}, regardless of melatonin concentration. Exogenous application of melatonin mitigated the effects of salt stress on chlorophyll b and relative water content at salinity level of 5.00 dS m^{-1} and concentration of 1 mM.

Keywords: *Glycine max*. Salinity. Mitigating. Bioregulator.

RESUMO - A salinidade é um fator abiótico que prejudica o crescimento e os mecanismos fisiológicos, bioquímicos e moleculares das plantas. Entre as plantas, a soja é uma cultura importante mundialmente, portanto, o manejo de fatores abióticos é essencial para mitigar os danos às plantas. No entanto, bioestimulantes, como a melatonina, estão sendo usados para aliviar o estresse causado por esses fatores. Portanto, este estudo teve como objetivo avaliar o crescimento, os pigmentos fotossintéticos e as relações hídricas de plantas de soja aplicadas a níveis de salinidade e aplicação exógena de melatonina. A pesquisa foi realizada em área experimental pertencente à Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brasil. O delineamento experimental foi em blocos casualizados, arranjados em esquema fatorial 3 x 3 (três níveis de salinidade na água de irrigação – 0.50, 3.00 e 5.00 dS m^{-1} e três concentrações de melatonina – 0, 0.5 e 1 mM), com três repetições. Aos 47 dias após o plantio, foram avaliados a altura da planta, diâmetro do caule, número de folhas, comprimento radicular, teor de clorofila (a, b e total), conteúdo relativo de água, umidade foliar e extravasamento de eletrólitos. As plantas de soja toleraram os efeitos da salinidade nos aspectos de crescimento, nos pigmentos fotossintéticos e nas relações hídricas até 3,00 dS m^{-1}, independentemente da concentração de melatonina. A aplicação exógena de melatonina mitigou os efeitos do estresse salino na clorofila b e no conteúdo relativo de água, no nível de salinidade de 5.00 dS m^{-1} e concentração de 1 mM.

Palavras-chave: *Glycine max*. Salinidade. Atenuante. Biorregulador.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops in the world (LIN et al., 2022). This crop has gained prominence due to its adaptability to different climatic zones and can be used to produce various foods and industrial products (RATNAPARKHE et al., 2022). Although soybean is cultivated all over the world, its production is concentrated in ten countries with a total of 338 million tons, equivalent to 96.94% of world production, and the largest producers are Brazil, followed by the USA, Argentina, China, India, Canada, Russia, Paraguay, Bolivia and Ukraine (FAOSTAT, 2022). However, its production is limited when the crop is subjected to adverse conditions, such as abiotic factors (MISHRA et al., 2021; RASHEED et al., 2022).

Abiotic factors include low and high temperatures, harmful radiation, pesticides, water deficit and excess, salinity, and other factors. These factors can lead to reversible and irreversible disturbances in the course of plant development and in the structures that build it, compromising metabolic processes that occur in the cell and causing changes in the physicochemical properties of cellular structures (STANIAK; SZPUNAR-KROK; KOCIRA, 2023). Among them, salt stress impairs plant growth and development, as well as physiological, biochemical, and molecular mechanisms, reducing dry mass, yield, leaf area, and stem, root and aerial length (ZÖRB; GEILFUS; DIETZ, 2019). In soybean, growth, yield, and quality are impaired at all stages of growth when the crop is subjected to stressful salinity conditions (LI et al., 2021; RAZA et al., 2023).

Under normal or stressful conditions, plant development is regulated by natural or synthetic substances (SOARES et al., 2022), which are essential because they induce oxidative metabolism, regulate plant development, and act as precursors of phytohormones, biosynthetics, and amino acids. These substances are known as bioregulators (ZULFIQAR; ASHRAF, 2021). Melatonin (MT, N-acetyl-5-methoxytryptamine) is a bioregulator that improves several physiological aspects of plants (ARNAO; HERNÁNDEZ-RUIZ, 2021), including vegetative growth and development, photosynthesis and osmoregulation (ion exchange, adjustments in osmotic and water potentials, in addition to favoring the regulation of different metabolic pathways of carbohydrates, lipids and among other compounds) (BUTTAR et al., 2020).

In the literature there are several works on salinity and soybean (LI et al., 2021; RASHEED et al., 2022); however, there are few studies using melatonin to mitigate salt stress (ZHANG et al., 2019) and under semi-arid conditions, and the ideal concentration to mitigate this stress is not known. Therefore, it is necessary to carry out a study to evaluate the development of soybeans under salt stress and use a bioregulator as a stress attenuator. Therefore, it is hypothesized that exogenous application of melatonin mitigates the adverse effects of salt stress on soybean plants. This study aims to evaluate the growth, photosynthetic pigments, and water relations of soybean plants subjected to salinity levels and exogenous application of melatonin.

MATERIAL AND METHODS

The soybean cultivar 'Sambaíba convencional' was cultivated from November to December 2023 in an open environment located in an experimental area belonging to the Department of Agronomic and Forestry Sciences of the Federal Rural University of the Semi-Arid Region (DCAF/UFERSA), in Mossoró, Rio Grande do Norte, Brazil (5º12′28"S, 37º19′04"W, 24 m altitude). The climate of the region is classified as hot and dry – BSh type, according to Köppen (ALVARES et al., 2013). The average temperature in the region is 27.4 °C, with an average relative humidity of 68.9% and an irregular annual rainfall, with an average of 673.9 mm (CLIMATE-DATA, 2021). The climatic data during the experiment were collected from the automatic meteorological station of Mossoró – INMET (Figure 1).

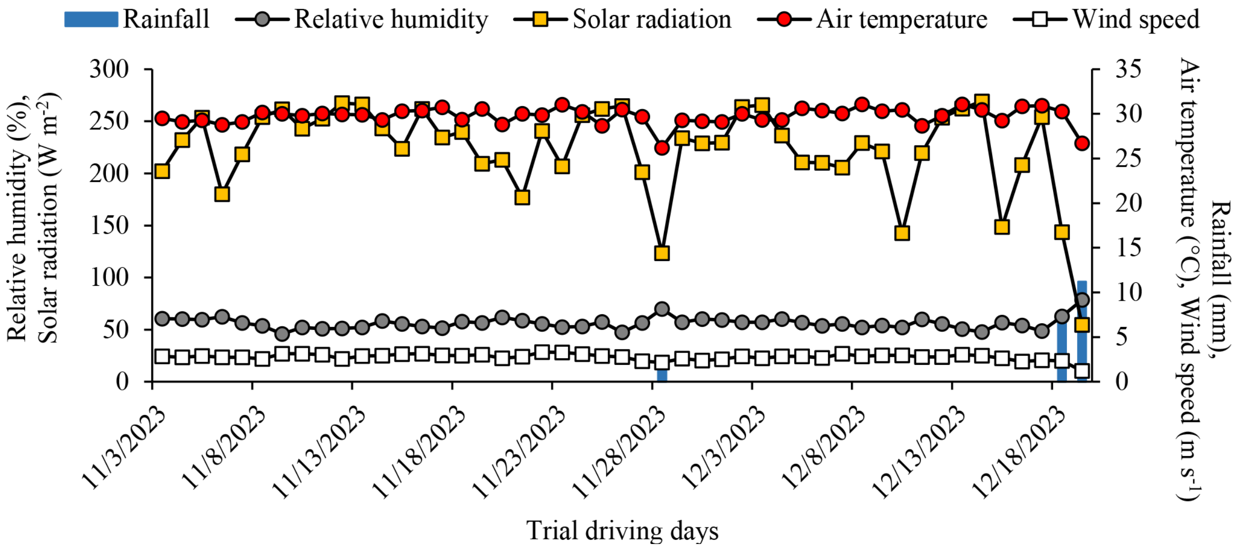


Figure 1. Meteorological data during the experimental period.

The experimental design was randomized blocks, arranged in a 3 x 3 factorial scheme (three salinity levels in irrigation water – 0.50, 3.00 and 5.00 dS m^{-1} and three melatonin concentrations – 0, 0.5 and 1 mM) with 3 replications. Salinity levels were obtained by adding sodium chloride (NaCl) to water to obtain the solutions, except for the 0.5 dS m^{-1} level (control – supply water) (Table 1).

Table 1. Chemical attributes of the supply water used in irrigation in the experiment.

pH	EC	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	CO_3^{2-}	HCO_3	SARse	Hardness	Cations	Anions
H_2O	(dS m^{-1})	--------------------------- $mmol_c$ L^{-1} ---------------------------							$(mmol\ L^{-1})^{0.5}$	mg L^{-1}	---- $mmol_c$ L^{-1} ----	
8.8	0.50	0.25	4.23	0.70	1.90	3.00	0.60	2.80	3.7	130	7.08	6.40

EC – Electrical conductivity; SARse – Sodium adsorption ratio in the saturation extract.

Soybean seeds were sown in 2.6 L polyethylene pots filled with sieved soil collected near the experimental area (Table 2), using five seeds per pot. Fertilization was carried out according to Gomes and Coutinho (2008), consisting of 40 and 60 kg ha^{-1}, respectively, of P_2O_5 and K_2O. The sources providing P and K were monoammonium phosphate (MAP) (61% P_2O_5 and 12% N) and potassium chloride (KCl) (60% K_2O). At 7 days after sowing (DAS), thinning was performed, selecting two plants per pot. At 15 days after sowing (DAS), the second thinning was carried out, leaving one plant per pot. The plants were irrigated daily with supply water until the beginning of the treatments. The control of invasive plants was performed manually throughout the experiment. At 28 DAS, the plants were subjected to salinity levels and exogenous application of melatonin.

Table 2. Physical and chemical analysis of the soil used in the experiment.

Soil depth	pH	EC	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	SB	t	T	V	m	ESP
(m)	(water)	dS m^{-1}	--------mg dm^{-3}--------			---------------cmol$_c$ dm^{-3}---------------					-------------%-------------			
0 – 0.20	7.11	0.06	104.7	177.4	16.4	3.20	1.10	0	4.83	4.83	4.83	100	0	1

Particle-size fractions					Textural class
--kg kg^{-1}--					
Coarse sand	Fine sand	Total sand	Silt	Clay	
0.48	0.33	0.81	0.14	0.05	Loamy sand

EC – Electrical conductivity of soil saturation extract; SB – Sum of bases; t – Effective cation exchange capacity; T – Cation exchange capacity; V – Base saturation; m - aluminum saturation; ESP - Exchangeable sodium percentage.

Melatonin solutions were obtained by dissolution in distilled water and were applied weekly, totaling 3 applications (28, 35 and 42 DAS). The leaves were sprayed using a spray bottle. Polysorbate 80 surfactant (Tween-80, 0.05% v/v) was added to melatonin concentrations to increase adhesion to the leaf.

At the end of the experiment (47 DAS), the following variables were evaluated: plant height, measured with a ruler graduated in centimeters (cm); stem diameter, measured with a digital caliper in millimeters (mm); number of leaves, counted manually; and root length, measured with a graduated ruler (cm). In the leaves, the contents of chlorophyll a, chlorophyll b, and total chlorophyll were also evaluated. These were estimated using a portable device (Clorofilog) on the middle third of the leaves, and reading was performed on two leaves; leaf moisture (SLAVICK, 1979); relative water content (IRIGOYEN; EINERICH; SÁNCHEZ-DÍAZ, 1992) and electrolyte leakage (LUTTS; KINET; BOUHARMONT, 1996). For the variables leaf moisture, relative water content and electrolyte leakage, ten leaf discs were used for each replicate, both measuring six millimeters.

The data obtained were subjected to analysis of variance (F test). Tukey's test was performed at 5% probability level for comparison of means in cases of significance. The variables were analyzed using the statistical program Sisvar 5.6 (FERREIRA, 2014). Pearson's correlation and principal component analysis (PCA) were performed to verify the relationship between the analyzed variables.

RESULTS AND DISCUSSION

Salinity reduced height, diameter, and number of leaves at all salinity levels and melatonin concentrations. Plant height decreased with the intensification of salinity levels (Figure 2A). The concentration of 0.5 mM alleviated salt stress up to 3.00 dS m^{-1} for the number of leaves (Figure 2C). In addition, the number of leaves was reduced by 43.40% at the 1 mM concentration when comparing the 0.50 and 5.00 dS m^{-1} levels. At the 1 mM concentration, stem diameter showed a reduction of 28.86% between the salinity levels of 0.50 dS m^{-1} and 5.00 dS m^{-1} (Figure 2B). There was no significant difference in root length, with an average value of 26.40 (Figure 2D).

Plant growth restriction is one of the first reactions to stress caused by water scarcity or salinity, resulting in morphological, physiological, and biochemical changes (LEE et al., 2016). These changes in morphology can be seen in Figure 3.

Most cultivated plants have impairment in physiological processes due to salinity. As a result, salt stress affects them through its osmotic effects, causing damage that favors deficient growth and development (HERNÁNDEZ, 2019; KAMRAN et al., 2019). Considering this, prolonged exposure to salt stress reduces biomass production, height, number of leaves, root length, and other parameters, mainly due to the interference of salts with the plant's internal water content (ACOSTA-MOTOS et al., 2017). In contrast, melatonin is a substance that promotes plant growth, photosynthesis, as well as the accumulation of antioxidants under stressful environmental conditions (ZHANG et al., 2019). Therefore, the influence of salt stress on soybeans was evident in the present study. A similar result was observed by Rady et al. (2019), who studied species from the same family and identified a negative correlation between biometric parameters and salinity in common beans.

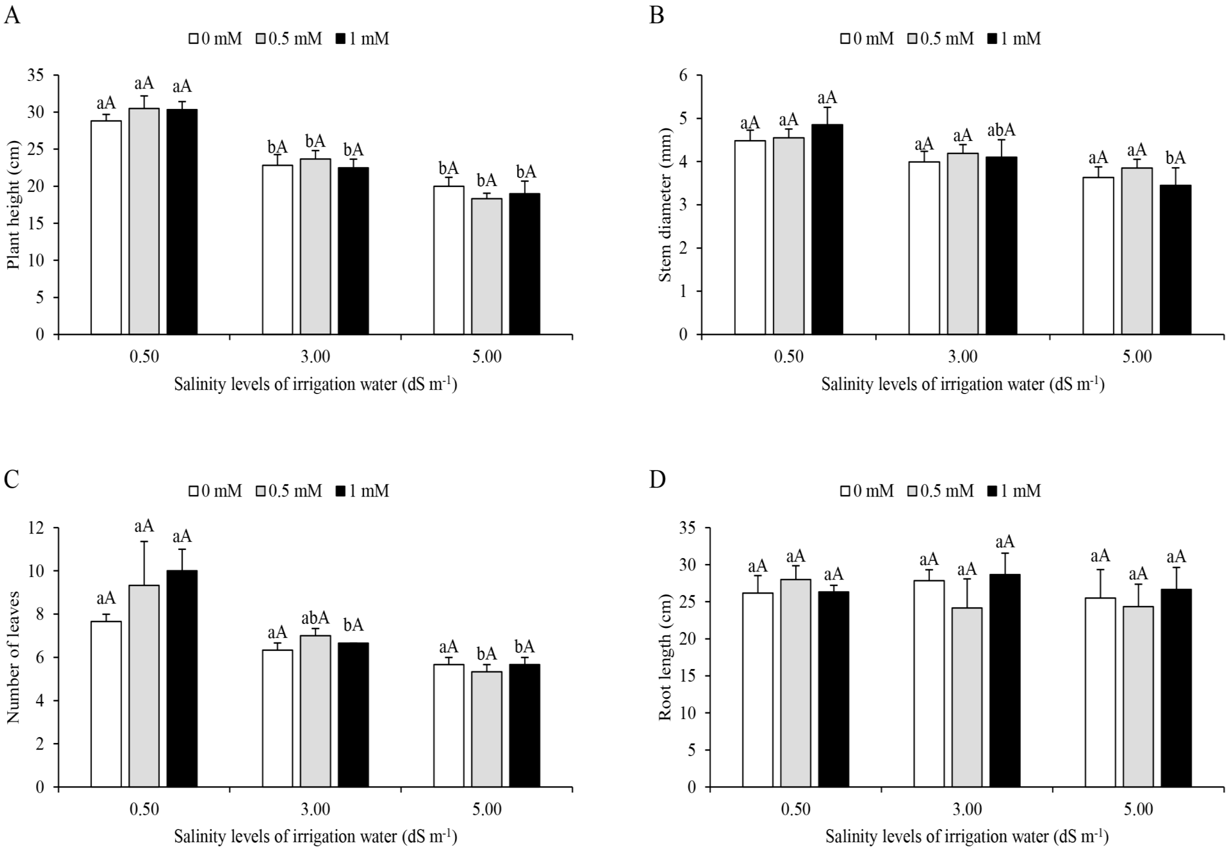


Figure 2. Plant height – (A), stem diameter – (B), number of leaves (C) and root length – (D) of soybean plants subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and under exogenous application of melatonin (0, 0.5 and 1 mM). Lowercase letters differ in the salinity levels of irrigation water, and uppercase letters differ in melatonin concentrations ($p<0.05$). Bars show the standard error of the mean.

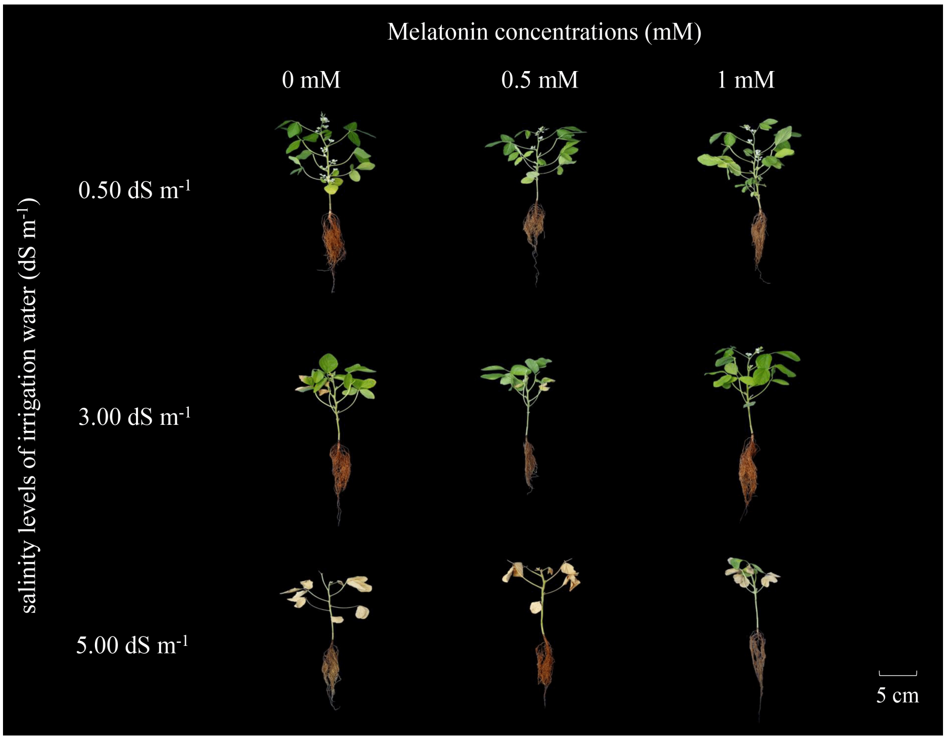


Figure 3. Soybean plants, cultivar 'Sambaíba convencional', subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and exogenous application of melatonin (0, 0.5 and 1 mM).

Chlorophyll a, chlorophyll b and total chlorophyll contents significantly decreased at a melatonin concentration of 0.5 mM at a salinity level of 5 dS m^{-1}, showing reductions of 39.21, 53.46 and 42.97%, when compared to the salinity level of 0.50 dS m^{-1} (Figure 4). For chlorophyll b, the 1 mM concentration caused an increase of 38.69% at the salinity level of 5 dS m^{-1}, when compared to the 0.5 mM concentration at the same salinity level. Photosynthetic pigments, such as chlorophyll a and b, play a crucial role in energy absorption and transfer in the complex photosynthetic process, so these pigments are closely related to the metabolism and photochemical events of photosynthesis (KHATRI; RATHORE, 2022).

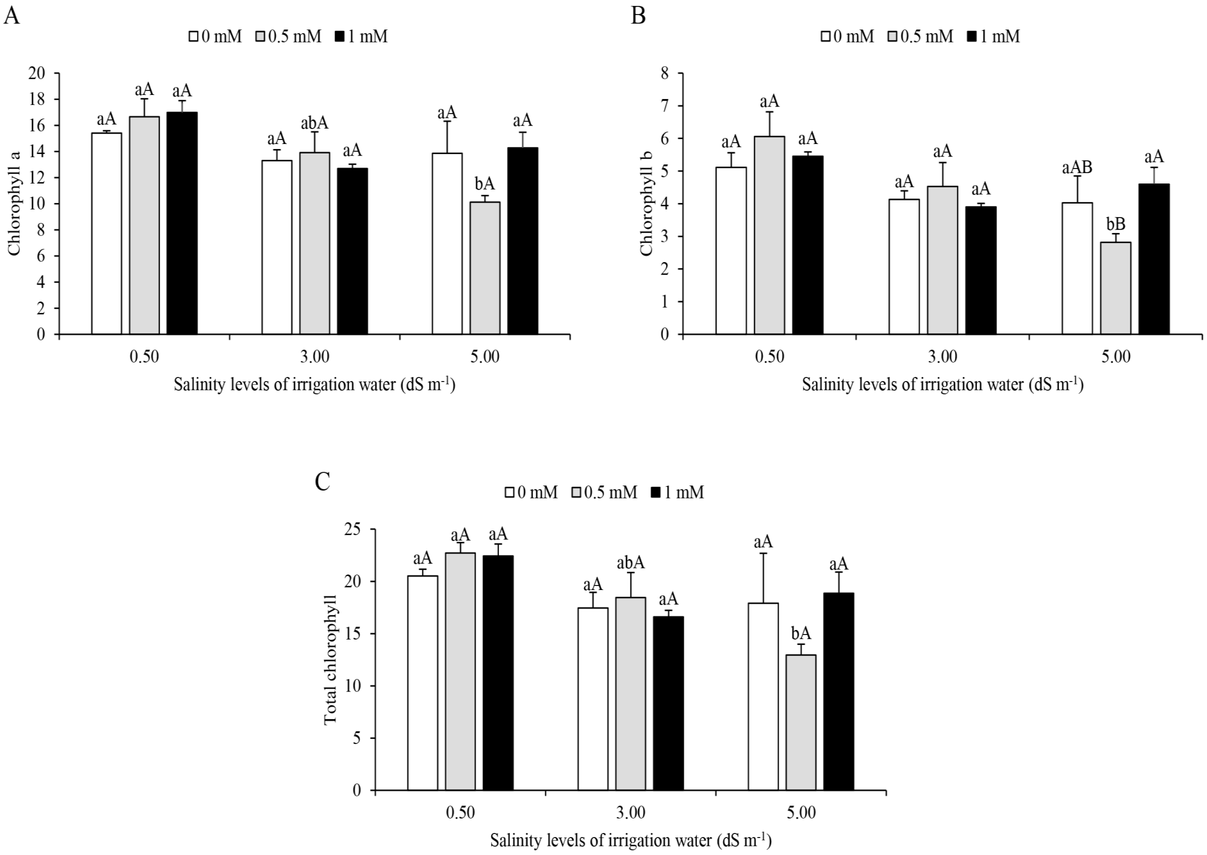


Figure 4. Chlorophyll a - (A), chlorophyll b - (B) and total chlorophyll (C) of soybean leaves subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and exogenous application of melatonin (0, 0.5 and 1 mM). Lowercase letters differ in the salinity levels of irrigation water, and uppercase letters differ in melatonin concentrations ($p<0.05$). Bars show the standard error of the mean.

A justification for the reduction of chlorophyll b is that melatonin is critical in integrating signals from biotic and abiotic stresses, favoring defense mechanisms. Melatonin can act in signaling, initiating processes to mitigate stress, and also in stress tolerance, increasing the expression of genes related to cell division, photosynthesis, and osmolyte synthesis (WANG et al., 2023). Therefore, it is inferred that, at a concentration of 0.5 mM, melatonin may have been redirected to signaling processes, while at the concentration of 1 mM it was critical and translocated to defense mechanisms, favoring plant tolerance. These are adaptive responses to the imposed condition.

There was a significant interaction between salinity and melatonin concentration. The relative water content decreased with the increase in salinity levels at a concentration of 0.5 mM, showing reductions of 56.94% and 55.92% at the salinity level of 5.00 dS m^{-1}, when compared to the salinity levels of 0.50 and 3.00 dS m^{-1}, respectively (Figure 5A). The concentration of 1 mM improved the relative water content at the salinity level of 5.00 dS m^{-1}, showing an increase of 32.52% compared to the concentration of 0 mM at the same salinity level.

Leaf moisture increased as salinity levels increased at the 0 mM concentration, with 32.72 and 18.54% increments for salinity levels 3.00 and 5.00 dS m^{-1}, respectively, compared to the 0.50 dS m^{-1} level (Figure 5B). Melatonin concentrations did not influence leaf moisture at the salinity levels studied. These variables are essential parameters, as they show in the short term the change in water content under stressful conditions, so it is clear to observe that the increase in salinity reduces the relative water content of the tissues and, consecutively, the potential for cell turgidity, leading to the reduction of leaves and roots, as well as severe damage in the cell plasmolysis stage (SHARIF et al., 2018).

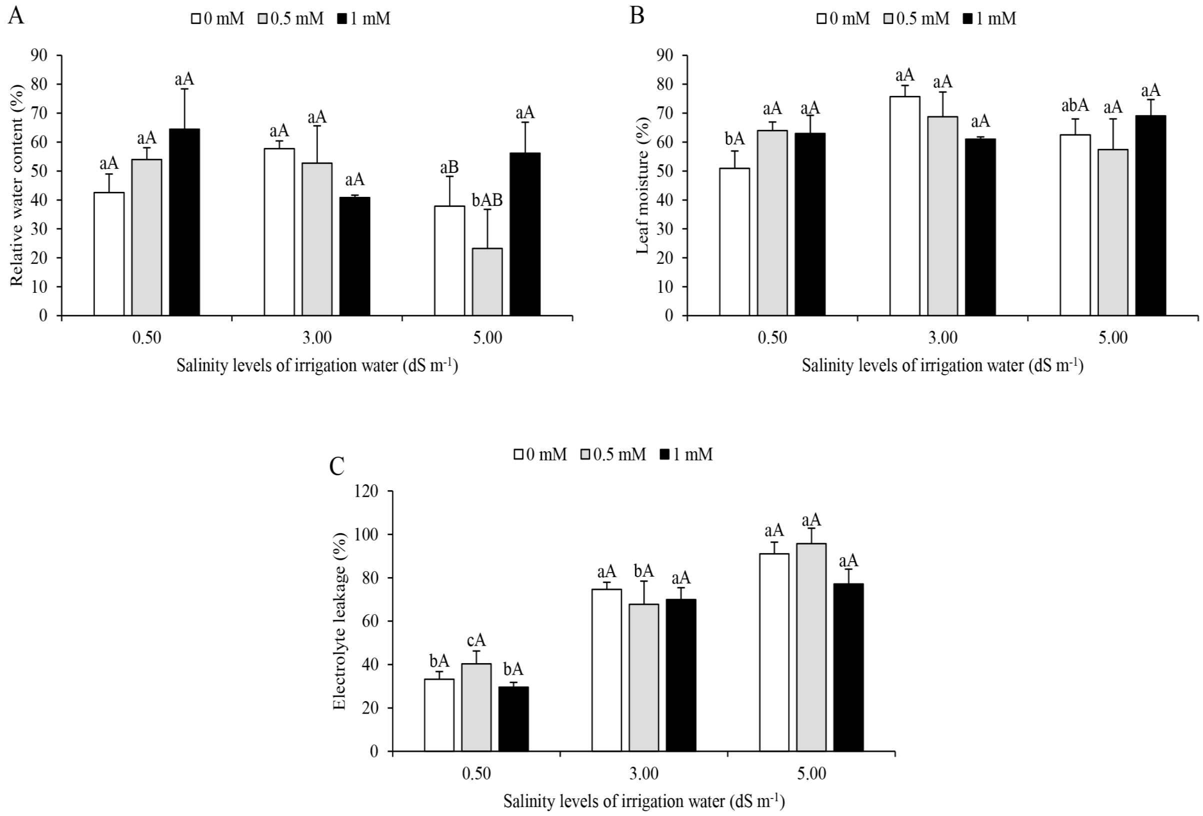


Figure 5. Relative water content - (A), leaf moisture - (B) and electrolyte leakage - (C) in leaves of soybean subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and under exogenous application of melatonin (0, 0.5 and 1 mM). Lowercase letters differ in the salinity levels of irrigation water, and uppercase letters differ in melatonin concentrations ($p<0.05$). Bars show the standard error of the mean.

There was a significant difference only for the salinity factor regarding electrolyte leakage (Figure 5C). Electrolyte leakage was intensified as salinity levels increased, causing membrane damage of 33.24, 74.59, and 91.04%, respectively, at 0.50, 3.00, and 5.00 dS m^{-1} for the 0 mM concentration. At the concentrations of 0.5 and 1 mM, the most significant damage was observed at the salinity level of 5 dS m^{-1}, with 95.74 and 77.09% values in the same order. This occurs because salinity causes osmotic stress, hindering the functions of the biomembrane; in addition, this osmotic dysregulation causes ionic toxicity, leading to the production of reactive oxygen species followed by oxidative damage (ALHARBY et al., 2021).

The sum of the principal components (PC), PC1 and PC2, resulted in a total inertia of 85.40% of the total variation (Figure 6). PC1 contributed with 67.90% to the total variation and obtained positive correlations with the variables plant height (PH), stem diameter (SD), number of leaves (NL), chlorophyll a (Chlo a), chlorophyll b (Chlo b), total chlorophyll (T chlo), relative water content (RWC) and root length (RL) for 0.50 EC and at both concentrations. PC2 contributed with 17.50% to the total variation and obtained a negative correlation with electrolyte leakage (EL), since EL showed inverse behavior to the variables PH, SD and NL, demonstrating that membrane damage negatively influences plant height, stem diameter and number of leaves. In addition, LM showed a different behavior from the other variables, while 5.00 EC + 0.5 mM did not correlate with any variable.

EL showed strong negative correlations with PH (-0.96), SD (-0.89), NL (-0.92), Chlo a (-0.85), Chlo b (-0.86) and T chlo (-0.86), and weak and moderate negative correlations with RL (-0.31) and RWC (-0.57), respectively (Figure 7). These associations demonstrate that increased electrolyte leakage causes reductions in the variables above and vice versa. In addition, good growth is related to the state of the physiological aspects of the plant, since PH showed strong positive correlations with Chlo a (0.84), Chlo b (0.86) and T chlo (0.85).

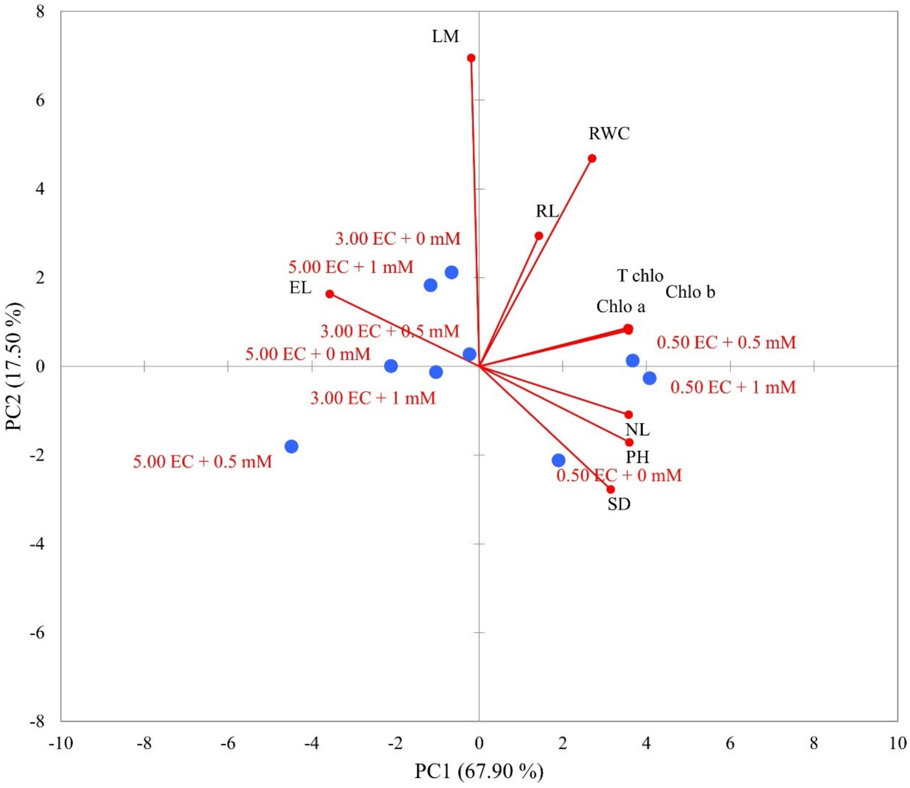
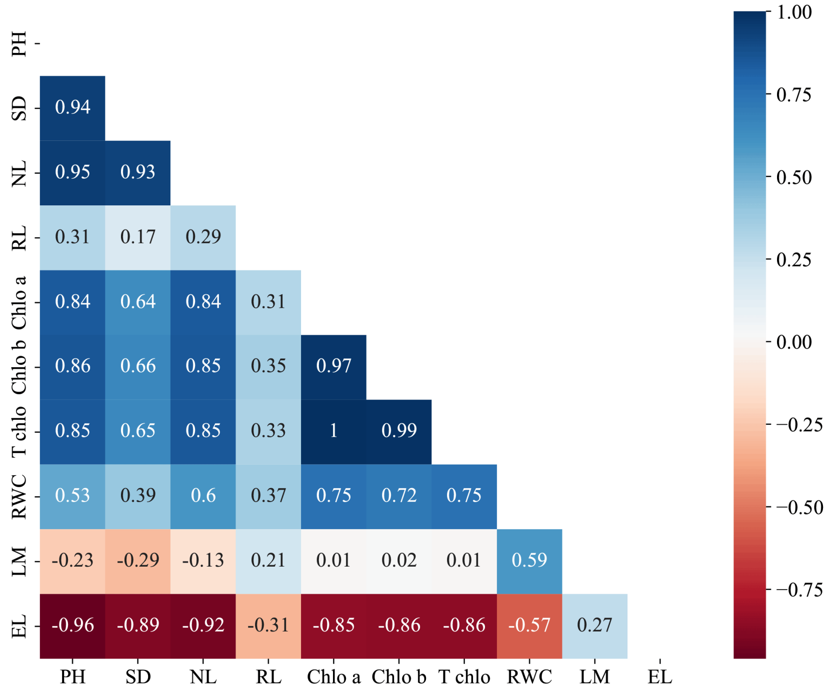


Figure 6. Principal Component Analysis (PCA) of growth variables (PH – Plant height; SD – Stem diameter; NL – Number of leaves; RL – Root length), physiological aspects (Chlo a – Chlorophyll a; Chlo b – Chlorophyll b; T chlo – Total chlorophyll) and water relations (RWC – relative water content; LM – Leaf moisture; EL - Electrolyte leakage) of soybean plants subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and under exogenous application of melatonin (0, 0.5 and 1 mM).



	PH	SD	NL	RL	Chlo a	Chlo b	T chlo	RWC	LM	EL
PH										
SD	0.94									
NL	0.95	0.93								
RL	0.31	0.17	0.29							
Chlo a	0.84	0.64	0.84	0.31						
Chlo b	0.86	0.66	0.85	0.35	0.97					
T chlo	0.85	0.65	0.85	0.33	1	0.99				
RWC	0.53	0.39	0.6	0.37	0.75	0.72	0.75			
LM	-0.23	-0.29	-0.13	0.21	0.01	0.02	0.01	0.59		
EL	-0.96	-0.89	-0.92	-0.31	-0.85	-0.86	-0.86	-0.57	0.27	

Figure 7. Pearson's correlation between growth variables (PH – Plant height; SD – Stem diameter; NL – Number of leaves; RL – Root length), physiological aspects (Chlo a – Chlorophyll a; Chlo b – Chlorophyll b; T chlo – Total chlorophyll) and water relations (RWC – relative water content; LM – Leaf moisture; EL - Electrolyte leakage) of soybean plants subjected to salt stress (0.50, 3.00 and 5.00 dS m^{-1}) and under exogenous application of melatonin (0, 0.5 and 1 mM).

CONCLUSIONS

Soybean plants tolerated the effects of salinity on growth aspects, photosynthetic pigments, and water relations up to 3.00 dS m^{-1}, regardless of melatonin concentration.

Exogenous application of melatonin mitigated the effects of salt stress on chlorophyll b and relative water content at salinity level of 5.00 dS m^{-1} and concentration of 1 mM.

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